Comparative effects of furnished and battery cages on egg production and physiological parameters in White Leghorn hens

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ABSTRACT Laboratory animal well-being can be improved by housing the animals in species-specific natural or near-to-natural environments. An enriched environment may have a similar effect on chickens. The purpose of this study was to examine if housing environment (furnished cages vs. battery cages) effects the well-being of laying hens. One hundred ninety-two 1-d-old non-beak-trimmed White Leghorn W-36 chicks were reared and randomly assigned into battery cages or furnished cages at 19 wk of age. The furnished cages had wire floors and solid metal walls, with perches, a dustbathing area, scratch pads, and a nestbox area with concealment curtain. Ten hens were housed per cage, providing a stocking density of 610 cm^2 of floor space per hen. The battery cages were commercial wire cages containing 6 birds per cage, providing 645 cm^2 of floor space per hen. Body weight and egg production were calculated from 25 to 60 wk of age. The peripheral concentrations of dopamine, epinephrine, norepinephrine, serotonin, corticosterone, and IgG were analyzed at 30, 40, 50, and 60 wk of age. Compared with the hens housed in the battery cages, the hens housed in the furnished cages were significantly heavier from 30 to 60 wk of age ($P < 0.05$ and 0.01, respectively) and produced more eggs at 40 wk of age ($P < 0.05$). There were no treatment effects on eggshell thickness ($P > 0.05$). The concentrations of serotonin were reduced, whereas corticosterone was increased from 50 to 60 wk of age in the hens housed in the battery cages ($P < 0.05$) but not in those housed in the furnished cages, which may indicate that the hens housed in the battery cages were stressed. Although further studies remain to be completed, the present results suggest that furnished cages may be a favorable alternative for housing laying hens.

INTRODUCTION

Laying hens in the United States today are primarily housed in battery cages (also called conventional cages). The use of battery cages raises a considerable debate pertaining to the relative effect of the practice on hen well-being. Battery cages provide some benefits to the well-being of hens, such as maintaining a small stable group size, resulting in a low level of aggression and cannibalism, high egg production, and hygiene (Appleby, 1998; Rodenburg et al., 2005; Vits et al., 2005a). However, there is a considerable body of morphological, physiological, and behavioral evidence demonstrating that the use of battery cages increases stress in hens due to an overcrowded, barren environment, which can inhibit the hens from performing certain natural behaviors and reduce bone quality (Hughes et al., 1993; Nicol, 1995; Vestergaard et al., 1997; Tauson, 1998). There is growing pressure from animal well-being and consumer groups advocating a global ban of battery cage systems. The poultry producers and scientists are in a prime position to preempt any future legislative restriction of battery caging systems by evaluating its effects on hen well-being and implementing more welfare-friendly housing systems that minimize stress and safeguard hen well-being.

Currently, researchers are examining the effect of various laying hen housing systems on bird welfare (Cunningham and Mauldin, 1996; Appleby et al., 2002; Dawkins et al., 2004; Tauson, 2004; Mertens et al., 2006; Miller and Mench, 2006). Furnished cage systems attempt to provide an enriched environment (i.e., facilities) to meet the needs of hens while maintaining small group size to minimize social stress (Tauson, 1998). Furnished cages are equipped with perches, dustbath areas, and nesting areas, to increase opportunities for the hens to exhibit natural behaviors (Lindberg and Ni-

col, 1997; Appleby et al., 2002). Previous studies have shown that furnished cages also improve hen well-being by reducing fear, aggression, and feather pecking, and increasing bone mineral density (Fleming et al., 1994; Gvaryahu et al., 1994; Newberry, 1995; Kopka et al., 2003; Leyendecker et al., 2005; Vits et al., 2005b). Although furnished cage systems may improve hen well-being, influences have been shown to be strain-, age-, and facility-dependent. Before recommending its widespread use within the US egg industry, a full-scale scientific evaluation of the purposed benefits of furnished cages is necessary.

Environmental enrichment induces various changes in physiology and behavior in humans and other mammals, which, in turn, affects their physical and psychological well-being (Spires and Hannan, 2005; Nithianantharajah and Hannan, 2006; Baker et al., 2007; Segovia et al., 2008). Among various hormones and neurotransmitters, corticosterone (**CORT**), epinephrine (**EP**), norepinephrine (**NE**), dopamine (**DA**), and serotonin (**5-HT**) play important roles in regulating the stress response to environmental stimuli in humans and rodents (Kingston and Hoffman-Goetz, 1996; Manuck et al., 2005; Meijer et al., 2007; Bean et al., 2008; Brenes et al., 2008; Segovia et al., 2008). Previous studies have shown that the avian neuroendocrine system responds to stimuli similar to mammalian systems (Harvey et al., 1984). In addition, immunity, such as producing antibody IgG, is affected by social environments (Cunnick et al., 1991; Tuchscherer et al., 1998). We hypothesize that changes in the endogenous levels of these compounds may underlie the differential reactions of hens to the furnished cages and battery cages. The objective of this study was to determine the effects of cage systems, furnished cages versus battery cages, on production and physiological parameters of White Leghorn hens.

MATERIALS AND METHODS

Chickens and Housing Systems

One hundred ninety-two, 1-d-old non-beak-trimmed Hy-Line W-36 White Leghorn female chicks were reared following standard management practices in raised wire cages. At 19 wk of age, the pullets were randomly assigned to 1 of 2 different housing treatments: battery cages or furnished cages (12 cages/treatment). The battery cages ($102 \times 38 \times 46$ cm; length $\times$ width $\times$ height) were commercial wire cages containing 6 hens per cage, providing 645 cm^2 of floor space/hen. For comparison, attempts were made to use a comparable stocking density in the furnished cages. The furnished cages ($120 \times 55 \times 45$ cm; length $\times$ width $\times$ height; EV 550-EU, Big Dutchman, Vechta, Germany) had wire floors and solid metal walls, with perches arranged in front of the litter bath, a dustbathing area located at the left rear corner, scratch pads behind the feed trough, and a nest-

box area with concealment curtain located at the right rear corner (Figure 1). Sand was used as a dustbathing substrate. The birds could access the facilities without restriction. Based on the company recommendations, 10 hens were housed per cage, providing a stocking density of 610 cm^2 of floor space/hen. Feed and water were provided ad libitum to both treatments. Overhead lights were on a 16L:8D schedule, from 0700 to 2300 h. Both housing treatments were located within the same room at Purdue University Poultry Farm.

Daily inspections were conducted by the Purdue University Poultry Unit staff to observe for body injury and mortality. The experimental protocols were approved by the Institutional Animal Care and Use Committee at Purdue University.

BW

Body weights of the sampled birds were taken immediately after blood collection at 30, 40, 50, and 60 wk, respectively. All weights were taken to the nearest gram.

Egg Production

Eggs were collected daily starting at 30 wk of age. All of the cages were cleaned up daily (i.e., no eggs were left) and all eggs were counted at the end of collection day. Egg weights were calculated starting at 30 wk of age by examining the eggs produced on the collection day (Monday) of each production week up to 60 wk of age. The egg weight was presented as average grams per egg for each cage, which was calculated as the following formula:

$$\text{Egg weight/egg} =$$
$$\text{total egg weight/total number of eggs.}$$

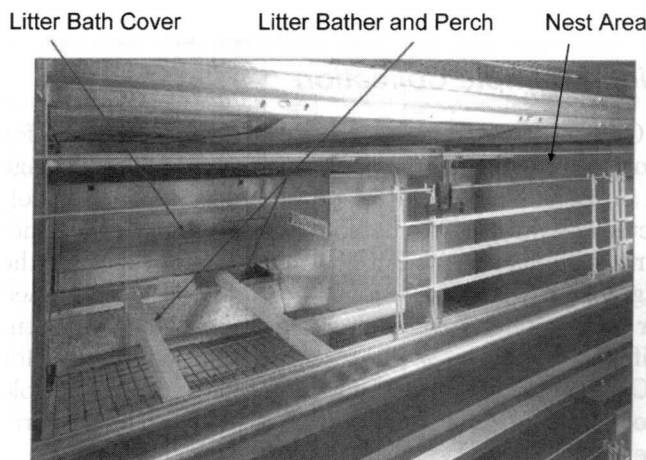Litter Bath Cover   Litter Bather and Perch   Nest Area

Figure 1. An illustration of distribution of the facilities (i.e., dustbathing area, perches, scratch pads, and nestbox area) within a furnished cage.

Shell thickness was calculated at 50 and 60 wk of age following the previously described protocols (Schreiweis et al., 2006; Jendral et al., 2008). Initial egg weights were obtained, then the eggs were cracked at the equator and the yolk and albumin were discarded. The empty shell was rinsed with tap water to remove the remaining albumin. Shells were then placed in an oven at 65°C for 12 h. After drying, shell thickness was measured using an Ames micrometer (model 25, BC Ames Company, Waltham, MA). Three thickness measurements were taken at the equator, 2 at the point end, and 2 at the blunt end using the micrometer. The mean shell thickness of the 7 measurements was calculated for each egg sampled (n = 12).

Feed Efficiency

Feed efficiency analysis was carried out at 55 and 60 wk of age using a 3-d protocol modified from those used by Paterson et al. (2000) and Yoruk et al. (2004). Trough feeders were emptied before the test. During the test, plastic trays were placed individually under feed troughs of each cage to collect wasted feed. A weighed portion of feed was added daily to the troughs. During each of the feed efficiency trials, hens had sufficient feed to consume ad libitum. At the end of the test, feed remaining in the feed troughs was weighed and plastic trays were removed from under the troughs. Plastic trays were removed and allowed to dry within the same room as the hens were housed. After the trays had dried, manure was separated from the waste feed using a sieve, and remaining feed waste was weighed. Daily egg production was documented on an eggs per cage basis for the 3-d period. Feed efficiency was calculated using the following formula:

$$\text{Grams of feed/egg} =$$
$$\frac{\text{Total grams of feed} - \text{Grams of feed wasted and left over/cage}}{\text{Number of eggs produced/cage}}.$$

Blood Sample Collection

One bird was randomly selected from each cage for blood sample collection at 30, 40, 50, and 60 wk of age (n = 12/treatment). Five milliliters of blood was collected into an EDTA-coated tube via jugular vein puncture from the hens within 2 min of removal from the cage. An aliquot of 500 µL of whole blood was retained for 5-HT analysis. The remainder of the blood was centrifuged at 700 × g for 15 min to obtain plasma for CORT, catecholamine, and IgG (IgY) analysis. Whole blood and plasma were kept at −80°C until measurements.

HPLC Assay

Plasma DA, EP, and NE were measured using a commercial catecholamine analysis kit (ESA Inc., Chelms-

ford, MA) following a previously described protocol (Cheng et al., 2001a). Duplicate plasma samples were acidified and deproteinized with 4 M perchloric acid. After centrifugation, the acid supernatants and internal standard dihydroxybenzylamine were added and absorbed onto an alumina minicolumn to bind the catecholamine. The columns were then rinsed and eluted with the solutions supplied by the company. After injection of eluents into the reverse-phase columns, catechols were detected with Coulochem II electrochemical detectors (ESA Inc.) by liquid chromatography. The mobile phase (75 mm of sodium phosphate, 1.7 mm of 1-octanesulfonic acid, 25 µm of EDTA, 10% acetonitrile, and 100 µL/L of triethylamine, adjusted to pH 3.00 with phosphoric acid) flow rate was 1.3 mL/min. The concentrations of DA, EP, and NE were calculated from a reference curve made using supplied standards and were presented in nanograms per milliliter.

For analysis of blood 5-HT levels, whole blood was acidified with 3% ascorbic acid, then deproteinated with 4 M perchloric acid. After centrifugation, the supernatants were filtered through a 0.2-µm filter. Samples were then analyzed using HPLC and the concentrations of 5-HT and tryptophan were calculated from a reference curve made using supplied standards.

RIA for CORT

Total plasma CORT was measured using a commercially available ^{125}I CORT RIA kit (MP Biomedicals, Solon, OH) as outlined by Cheng et al. (2001a). Briefly, to validate for parallelism and recovery in birds, adjustments of dilution to 1 to 5 were made (i.e., 20 µL of sample to 80 µL of steroid diluents). The concentration of CORT was calculated from a reference curve that ranges from 0.1 ng/mL (95.4% binding) to 4.0 ng/mL (14.9% binding), and the correlation coefficient was 0.9995. A well recovery of exogenous CORT was determined by adding known amounts of unlabeled CORT to aliquots of steroid diluent to produce theoretical concentrations of 0.5, 1.0, and 2.0 ng/mL, which yielded recovered concentrations of 0.48, 1.08, and 1.97 ng/mL, respectively. The sensitivity of the assay was 0.02 ng/mL. To limit technical effect on the data collection, all samples within the experiment were performed at the same time, and within- and between-assay CV were 7.6 and 9.8%, respectively.

ELISA for Concentrations of Plasma IgG

Plasma IgG levels were measured utilizing the chicken IgG ELISA quantitative kit (Bethyl Laboratories Inc., Montgomery, TX; Cheng et al., 2001b) following the provided manufacturer protocol. A 96-well plate was coated with goat anti-chicken IgG-Fc diluted to 1% with a coating buffer (0.05 M sodium carbonate, pH 9.6) at 100 µL per well and incubated at room temperature for 60 min. After washing, the wells were filled with 200 µL of blocking post coat solution (50 mM

Tris, 0.14 M NaCl, 1% BSA, pH 8.0) and incubated at room temperature for 30 min. After washing, the diluted samples and standards were loaded in duplicate in 96-well plates, respectively. After 1 h of incubation and washing, goat anti-chicken IgG (1:1,250 dilution) was added to each well of the plate and incubated for 60 min in the dark. After washing, the immunoperoxidase bridge was completed by using Bethyl TMB Peroxide Substrate and Peroxidase Solution B Kit (Cheng et al., 2001b) and was incubated for 30 min. The reaction was read using a microtiter plate reader at a wavelength of 450 nm utilizing the KC4 software package. Concentrations of IgG were calculated from the standards and presented as milligrams per milliliter, adjusted for the dilution of the initial plasma sample.

Statistical Analysis

Data were analyzed using a GLM in SAS Version 8.0 (SAS Institute Inc., Cary, NC). Model statements for data analysis included age, housing treatment, and the interaction between age and treatment. Data were tested for normality and corrected for normality if necessary, dependent on individual data sets. Where significant F-values were noted, appropriate post hoc tests (Tukey's) were performed to determine differences. A significant difference was at $P < 0.05$.

RESULTS

Bird Health

One bird housed in a battery cage died during the experiment. One bird housed in a furnished cage had a feed impaction on the side of its beak, which was treated with Nolvasan (Fort Dodge, Overland Park, KS) and healed. Six birds housed in the furnished cages (none in the battery cages) had bumblefoot, which was treated with triple antibiotic ointment and healed.

BW

Body weight was significantly affected by age and treatment (Table 1). The heaviest BW was at 60 wk of age in both groups, but the hens housed in the furnished cages had significantly heavier BW than those housed in the battery cages at all time points (i.e., 30, 40, 50, and 60 wk of age; Table 1, $P < 0.05$ and 0.01, respectively).

Egg Production

Compared with the hens housed in the battery cages, the hens housed in the furnished cages produced more eggs at 40 wk of age ($P < 0.05$). Hens housed in the furnished cages reached a peak egg production earlier than those housed in the battery cages [i.e., 40:50 wk of age (furnished cage:battery cage)]. The peak was not significantly reduced in the hens housed in the fur-

Table 1. Effect of cage system, furnished cages versus battery cages, on hen production[1]

Item	Battery cage				Furnished cage			
	30 wk	40 wk	50 wk	60 wk	30 wk	40 wk	50 wk	60 wk
Egg/hen per day	0.78 ± 0.02[b]	0.73 ± 0.04[b,B]	0.87 ± 0.02[a]	0.75 ± 0.01[b,A]	0.80 ± 0.01[b]	0.89 ± 0.02[a,A]	0.85 ± 0.04[b]	0.70 ± 0.01[c,B]
Egg weight (g)	54.27 ± 0.45[c]	60.71 ± 0.85[a]	58.03 ± 0.42[b]	60.24 ± 0.37[a]	55.63 ± 0.60[c]	60.64 ± 0.24[b]	60.09 ± 0.25[b]	62.92 ± 0.56[a]
BW (kg)	1.42 ± 0.04[c,A]	1.50 ± 0.05[b,A]	1.61 ± 0.05[a,A]	1.66 ± 0.06[a]	1.53 ± 0.04[c,C]	1.64 ± 0.04[b,C]	1.69 ± 0.04[a,B]	1.71 ± 0.04[a]

[a,b; a,c; b,c]Significant difference within the treatment ([a,b; b,c]$P < 0.05$; [a,c]$P < 0.01$).

[A,B; A,C]Significant difference between treatments ([A,B]$P < 0.05$; [A,C]$P < 0.01$).

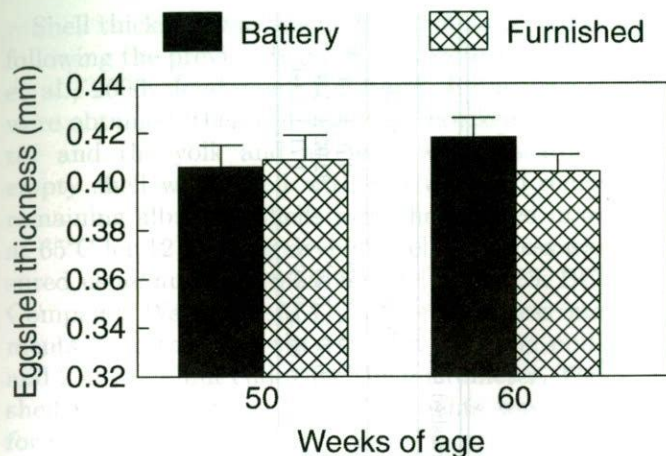[1]The data are presented as mean ± SE (n = 12).

Figure 2. The effects of housing environments, furnished cages versus battery cages, on eggshell thickness. The analysis was conducted when the hens were 50 and 60 wk of age. The data are presented as mean ± SE (n = 12).

nished cages from 40 to 50 wk of age (Table 1). Egg production was reduced from 50 to 60 wk of age in both cage systems, but the hens housed in the battery cages produced more eggs then those housed in the furnished cages at 60 wk of age ($P < 0.05$).

Egg weight was significantly affected by age ($P < 0.05$) but not by the treatments from wk 30 to 60 (Table 1). Eggshell thickness was not significantly affected by the treatments from 50 to 60 wk of age ($P > 0.05$, Figure 2). Feed efficiency was not affected by the treatments from 55 to 60 wk of age ($P > 0.05$, Figure 3).

Physiological Parameters

Plasma concentrations of EP, NE, CORT, and IgG were differently affected by age (Table 2). In both groups, EP concentrations were significantly increased from 30 to 60 wk of age, with a peak at 60 wk (Table 2, $P < 0.05$ and 0.01), whereas NE levels were higher only
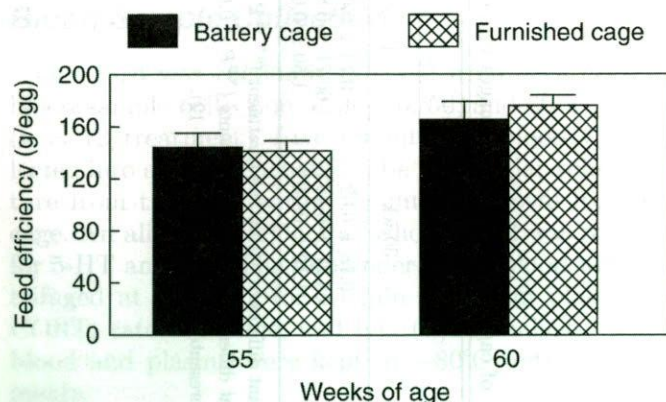

Figure 3. The effects of housing environments, furnished cages versus battery cages, on feed efficiency. The analysis was conducted when the hens were 55 and 60 wk of age. The data are presented as mean ± SE (n = 12).

at 60 wk of age compared with other examined time points ($P < 0.01$).

Dopamine concentrations were significantly affected by treatment and age (Table 2). Dopamine concentration was increased from 30 to 60 wk of age in both treatments. A peak increase in DA levels was found at 50 wk of age in the hens housed in the battery cages, followed by a reduction from 50 to 60 wk of age but still higher than the level at wk 30 and 40 ($P < 0.05$ and 0.01, respectively). Compared with the hens housed in the battery cages, the hens housed in the furnished cages had a delayed peak of DA concentrations that occurred at 60 wk of age.

There was no treatment effect on CORT concentrations among the hens ($P > 0.05$, Table 2), whereas age effects were found in the hens housed in the battery cages only ($P < 0.05$). In those hens, the CORT concentrations were increased with age, with a peak at 60 wk of age.

Plasma IgG concentrations were altered by age but not treatment (Table 2). In both groups, increased IgG concentrations were found at 60 wk of age compared with other observed time points ($P < 0.01$).

Plasma 5-HT concentrations were not affected by the treatments in the current study ($P > 0.05$, Table 2), whereas an age-related reduction of 5-HT concentration from wk 50 to 60 was found in the hens housed in the battery cages only ($P < 0.05$).

DISCUSSION

The Effects of Housing Environment on Hen BW Production

Body weight was greater in the hens housed in the furnished cages compared with those housed in the battery cages. A similar finding was reported by Schapiro and Kessel (1993). In that study, rhesus macaques housed in enriched environments had significantly greater BW compared with the controls that received no enrichment. In addition, Balog et al. (1997) reported that broilers housed in enriched environments with ramps had higher BW when compared with those housed in similar environments without ramps. Those authors indicated that the weight gain in enriched animals could be related to increased activities. The similar reason could be presented in the current study because the furnished cages, compared with the battery cages, provide more facilities for the hens to use.

The greater BW in the hens from furnished cages may be associated with increased bone density due to perch utilization in the furnished cages. Kopka et al. (2003) reported a positive correlation between BW and bone mineral density. Perch utilization and increased behavioral repertoire may increase bone density as well as the skeletal musculature. Jendral et al. (2008) also reported that perches increased total and cortical bone mineral density and breaking strength in the humerus

Table 2. Effects of cage systems, furnished cages versus battery cages, on the concentrations of neurohormones in hens[1]

Item	Battery cage				Furnished cage			
	30 wk	40 wk	50 wk	60 wk	30 wk	40 wk	50 wk	60 wk
Epinephrine (µg/mL)	0.39 ± 0.10[c]	0.93 ± 0.22[b]	1.27 ± 0.25[ab]	1.68 ± 0.23[a]	0.19 ± 0.07[c]	0.93 ± 0.14[b]	0.92 ± 0.12[b]	1.69 ± 0.20[a]
Norepinephrine (pg/mL)	0.40 ± 0.14[b]	0.72 ± 0.13[ab]	0.54 ± 0.20[b]	1.57 ± 0.26[a]	0.18 ± 0.09[b]	0.27 ± 0.11[b]	0.19 ± 0.05[b]	1.92 ± 0.26[a]
Dopamine (pg/mL)	0.18 ± 0.08[bc]	0.18 ± 0.05[bc]	1.05 ± 0.13[a,A]	0.41 ± 0.09[b,B]	0.09 ± 0.01[b]	0.14 ± 0.02[b]	0.71 ± 0.05[a,B]	0.96 ± 0.19[a,A]
Serotonin (µg/mL)	19.80 ± 1.83[ab]	19.83 ± 1.48[ab]	21.40 ± 1.45[a]	14.73 ± 0.04[b]	15.81 ± 2.40	18.54 ± 1.81	19.41 ± 1.77	15.29 ± 0.04
Corticosterone (µg/mL)	6.88 ± 1.06[b]	4.46 ± 1.13[b]	9.06 ± 1.26[ab]	11.45 ± 1.28[a]	6.92 ± 4.08	6.85 ± 1.58	9.71 ± 1.82	9.71 ± 1.82
IgG (mg/mL)	1.45 ± 0.26[b]	1.80 ± 0.20[b]	1.52 ± 0.27[b]	2.67 ± 0.14[a]	1.59 ± 0.28[b]	1.23 ± 0.71[b]	1.42 ± 0.19[b]	3.14 ± 0.30[a]

[a,b; a,c; b,c] Significant difference within the treatment ([a,b; b,c] $P < 0.05$; [a,c] $P < 0.01$).

[A,B] Significant difference between treatments ($P < 0.05$).

[1] The data are presented as mean ± SE (n = 12).

in birds. In addition, Barnett et al. (2009) reported that there was a benefit of perches on bone strength in laying hens. Those authors indicated that furnished systems promote load-bearing movement of birds, by which it not only preserves cortical structural bone loss but also simultaneously produces stronger bone.

In addition, the different BW gain between the hens housed in the different cages (furnished vs. battery cages) could be related to the different changes in the hormonal homeostasis, such as CORT and 5-HT. Both CORT and 5-HT are involved in regulating food intake and energy expenditure. Body weight was reduced in rats after chronic increases in hypothalamic-pituitary-adrenal activation, such as increased corticotrophin-releasing factor concentrations (Appel et al., 1991) or implantation of a slow-release CORT pellet (Calogero et al., 1991; Bush et al., 2003). Disorder of the serotonergic system, such as increases in 5-HT concentrations, may also associate with less BW gain in the hens housed in the battery cages. A similar correlation between the levels of 5-HT and BW was reported in rodents (Cai et al., 1995; Leibowitz and Alexander, 1998; Bush et al., 2003).

The Effects of Housing Environment on Egg Production

Environmentally enriched cages caused a left shift in the onset of peak production in hens (40:50 wk of age; furnished cage:battery cage). In general, there were no significant differences in egg production (egg/hen per day), although the hens housed in the furnished cages reached the peak of egg production earlier compared with the hens housed in the battery cages, 40 wk:50 wk (furnished cage:battery cage, Table 1). The egg weight (grams per egg), shell thickness, and feed efficiency were not different between the 2 housing systems. Early increases in production may be suggestive of reduced stress in furnished cage hens because the hens housed in the furnished cages had lower concentrations of both CORT and 5-HT compared with the hens housed in the battery cages. Both CORT and 5-HT have been used as stress indicators and directly and indirectly affect reproductive performances through binding to its receptors located in the central nervous system or reproductive organs in humans and animals including chickens (Tuomisto and Mannisto, 1985; Sirotkin and Schaeffer, 1997; Cheng et al., 2001a).

On the other hand, the egg production in the hens housed in the furnished cages could be further increased if all of the hens were kept at the same density and group size. Because of the limitation of the facilities, in the present study, the hens housed in the furnished cages were in a larger group size with a higher density compared with the hens housed in the battery cages, 10 hens/cage:6 hens/cage and 610 cm²:645 cm² (furnished cage:battery cage). The larger group size in the furnished cage treatment may reduce egg production

in laying hens as increasing competition. Craig et al. (1986) reported that chickens in a larger group size could be in a more socially stressed environment compared with those in a small group size, resulting in a lower egg production (Craig et al., 1986; Benyi et al., 2006). The effects of caging environments on productivity of hens deserve further studies with an equalizing group size and density of hens to eliminate the possibility of these environmental effects on egg productivity.

The Effects of Housing Environment on Physiological Parameters of Hens

The catecholamines, EP and NE, participate in many physiological and pathological processes, including regulation of emotion and motivation in response to stimuli (Elenkov and Chrousos, 2006). Changes in their concentrations have been used as indicators of well-being and ability to cope with stress in humans and rodents (Snider and Kuchel, 1983; Lehnert et al., 1984; Dimsdale and Ziegle, 1991; Wortsman, 2002; Morilak et al., 2005). The similar effects of those neuromodulators could be present in chickens. There is evidence that suggests that the stress regulation systems of birds are morphofunctionally homologous to the mammalians (Harvey et al., 1984; Palme et al., 2005)

In the present study, the concentrations of NE and EP were not affected by housing environments but were affected by aging, with a peak of both concentrations at wk 60. Similar to the present findings, age-related increases in NE and EP concentrations have been reported in rodents (Weiland et al., 1989; Kawano et al., 1995; Buchholz et al., 1998).

Dopamine, as a neurotransmitter or neuroendocrine modulator, is widely distributed in the central and peripheral nervous system in humans and other mammals (Lackovic and Relja, 1983; Hadjiconstantinou and Neff, 1987; Velasco and Luchsinger, 1998). Abnormalities in the blood and brain dopaminergic systems have been associated with dysfunctional behaviors as well as with declined coping ability with stress (Schneider, 1984; Kuikka et al., 1998). At 50 wk of age, DA levels in the hens housed in the battery cages were significantly higher than those housed in the furnished cages. In a previous study, it was shown that the hens selected for low group productivity and survivability had higher plasma DA concentrations than the counterparts selected for high group productivity and survivability when housed in the 10-bird cages (Cheng et al., 2003a,b), suggesting an important role for DA in the stress response in chickens. In that study, the hens of low group productivity and survivability and high group productivity and survivability were divergently selected based on egg production and mortality resulting from cannibalism and flightiness in colony cages (Cheng et al., 2001a). In addition, aggressive pecking, as a stressor in a social sitting, by dominant chickens

can be reduced by injection of raclopride, a DA receptor 2 antagonist (Dennis et al., 2006). The present and previous results indicate that DA can be used as an indicator for evaluating environmental stimulation.

Serotonin, as a neurotransmitter and neuromediator, is critical for maintaining adaptive, cognitive, and emotional processes in response to stimulation. Serotonin and its main metabolite, 5-hydroxyindoleacetic acid, have been used as indexes of stress in various animals (Steklis et al., 1986; Raleigh et al., 1991; Cubitt et al., 2008). Although the concentrations of 5-HT were not different in the hens between the treatments, they were reduced from 50 wk to 60 wk of age in the hens housed in the battery cages but not in those housed in the furnished cages. The reasons for this decrease of 5-HT concentrations are unclear, but it could be related to the different housing environments. In supporting the hypothesis, 5-HT concentrations did not decrease in the age-matched hens housed in the furnished cages, but the levels of CORT were increased in the hens housed in the battery cages only. In a comparison between the living conditions, the hens housed in the battery cages could be at a higher level of social stress compared with those housed in the furnished cages because they did not have facilities for performing certain behaviors. In supporting this hypothesis, 5-HT concentrations were reduced in stressed rodents and humans (Chaouloff et al., 1989; Takada et al., 1995). A previous study has found that CORT regulates 5-HT function in stress response (Lanfumey et al., 2008), and decreased plasma 5-HT concentrations were indicative of hyperactivity of the hypothalamic-pituitary-adrenal axis (Bianchi et al., 2002).

There was an age effect on CORT concentration found in the hens housed in the battery cage but not in those housed in the furnished cages. In the hens housed in the battery cages, the CORT concentration gradually increased from 40 to 60 wk of age. The changes of CORT concentrations could be related to age effects or social stress. Aging-associated elevated CORT concentrations have been found in humans and other animals (Wang et al., 1997; Kizaki et al., 2000). However, it is unlikely in the current study because, compared with the hens housed in the battery cages, the CORT concentrations were not changed in the age-matched hens housed in the furnished cages at the same room. Positive correlations between CORT response and social stress have been found in multiple species (Craig et al., 1986; de Kloet et al., 2008; Roelofs et al., 2009). The results may suggest that compared with hens housed in the furnished cages, the hens housed in the battery cages had a higher stress response.

It was found that IgG did not show treatment effects but had an increase from 50 to 60 wk of age in the hens from both treatments. The increase in IgG concentrations could be induced by aging. Age-associated hypergammaglobulinemia has been found in multiple species, including humans (Delespesse et al., 1977; Na-

kata et al., 2000; Weksler, 2000), nonhuman primates (Attanasio et al., 2001), pigs (Bianchi et al., 1999), and rodents (Senda et al., 1989; Zhao et al., 1995; Nobrega et al., 1996). In those studies, it has been shown that the increased IgG concentration could be related to 1) increases in the B lymphocytes in the circulating pool (Delespesse et al., 1977; Bianchi et al., 1999; Baumgarth et al., 2005) and 2) increases in autoantigens with aging (Nobrega et al., 1996; Attanasio et al., 2001). The high levels of total blood IgG might be released as a natural antibody, which, as Ehrlich (1906), Attanasio et al. (2001), and Baumgarth et al. (2005) hypothesized, was released without the necessity of previous interaction of the cells with antigen (in the complete absence of external antigenic stimulation). The functions of these antibodies might be related to the nonspecific immune response.

The present results suggest that different housing environments, furnished cages versus battery cages, affected differently the productivity and physiological homeostasis of hens. The data indicated that furnished cages could be a favorable alternative system for laying hens. However, the effects of caging environments on hen well-being deserve further studies with an equalizing group size and density of hens to eliminate the possibility of these environmental effects on hen stress response.

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